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Original research

Influence of tramadol on ischemia–reperfusion injury of rats' skeletal muscle

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HIGHLIGHTS

- The role of tramadol in ischemia–reperfusion injury is already proven.
- It has been shown that use of tramadol in myocardial or brain tissues decrease ischemia–reperfusion injuries.
- This study showed tramadol can alleviate the metabolic injuries in the skeletal muscle ischemia–reperfusion in this experimental model.

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ABSTRACT

Introduction: Tramadol has been shown to decrease ischemia–reperfusion injuries in myocardial or brain tissues. The aim of this study was to assess the effects of tramadol on ischemia–reperfusion injury in a rat hind limb ischemia–reperfusion model.

Methods: Forty-five healthy adult male Wistar rats were randomized into three experimental groups as follows: Sham, Ischemia–reperfusion and Ischemia–reperfusion + tramadol groups. Ischemia was induced in anesthetized rats by left femoral artery clipping for 2 h followed by 24 h of reperfusion. Tramadol (20 mg/kg) was administered intravenously immediately prior to reperfusion. Blood pH, pO_2, pCO_2, HCO_3, creatine phosphokinase (CPK), lactate dehydrogenase (LDH) as well as plasma malondialdehyde (MDA) were measured at the end of the reperfusion. Left gastrocnemius muscle samples were taken for histological and biochemical examination.

Results: The pH and pCO_2 were similar in all study groups, with no statistical significance. pO_2 and HCO_3 levels presented the highest elevation in sham and Ischemia–reperfusion + tramadol groups, as compared to Ischemia–reperfusion group ($P < 0.05$). The extent of muscle changes in the ischemia –reperfusion + tramadol group was significantly lower than ischemia–reperfusion group ($P < 0.05$). In comparison with other groups, serum and tissue MDA levels in ischemia–reperfusion group were significantly increased ($P < 0.05$). The muscle tissue glutathione (GSH), superoxide dismutases (SOD) and catalase (CAT) levels in the Ischemia–reperfusion group were significantly lower than the other groups ($P < 0.05$). Wet/dried weight ratio in ischemia–reperfusion group was significantly higher ($P < 0.05$) than subjects in other groups.

Conclusions: From the histological, histochemical and serum biochemical perspective, the treatment with tramadol has alleviated the metabolic injuries in the skeletal muscle ischemia and reperfusion in this experimental model.

1. Introduction

Ischemia in skeletal muscle is unavoidable in many vascular and muscular traumas, diseases and during a variety of surgeries in the upper or lower extremities. Re-establishment of blood flow can cause more extensive muscular injury than ischemia [1].

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After the re-introduction of oxygenated blood to ischemic tissues, free oxygen radicals are released and neutrophils are activated. This phenomenon is called as ischemia–reperfusion injury [2]. An acute inflammatory response is the basis of the physiopathology of ischemia–reperfusion injury. Organ dysfunction and finally organ failure can caused by complex mechanisms such as emerging free oxygen radicals and leukocyte aggregation [3,4].

Different types of protection and pharmacologic agents including antioxidants, vasodilators and specific peptide blockade of an injury-inducing IgM clone have been used to attenuate ischemia–reperfusion injuries in target or remote organ in clinical and experimental studies [5–7]. Factors that have been tested are superoxide dismutase, catalase, mannitol, hypertonic solutions, allopurinol, N-acetylcysteine, iron binding compounds, angiotensin converting enzyme inhibitors, calcium channel antagonists, alpha-tocopherol, zinc aspartate, oxytocin and Dexmedetomidine [8–16].

Tramadol hydrochloride is used as an effective analgesic for acute and chronic pain conditions, such as in cancer, neuropathic and post-operative pain. It has weak affinity to μ-opioid receptor and inhibits the reuptake of monoamines in the central nervous system thereby activating the descending inhibitory systems [17,18].

Recent researches disclose that tramadol has antioxidant effects, decreases lipid peroxidation and uses these therapeutic properties for the management of brain and lungs remote injuries after skeletal muscle ischemia–reperfusion [19,20].

Thus, considering the importance of acute arterial occlusion and its serious systemic consequences, an ischemia–reperfusion experimental study was performed in rats subjected to temporary clamping of the femoral artery, with the purpose of evaluating effects of tramadol on the histological findings present in skeletal muscles and biochemical changes secondary to muscle ischemia–reperfusion.

2. Material and methods

Forty-five healthy adult male Wistar rats, aged between 90 and 120 days with body weight between 250 and 350 g, were purchased from the Pasteur Institute of Iran. This study was conducted according to the guidelines of the animal care review board of the Islamic Azad University College of Veterinary Medicine and adhering to the guide for care and use of laboratory animals and the study is approved by the ethics committee. They were maintained under constant room temperature of 20–22 °C, relative humidity of 40–50%, 12 h/12 h light/dark cycle, with *ad libitum* access to water and commercial food.

2.1. Experimental groups

The rats were randomly allocated into three groups of fifteen rats each (of these 15, five were used for water content measurement, five for histochemical assays and five for histological analysis); Group 1 (Sham group) was subjected to all operative procedures, except arterial occlusion and reperfusion. The animals received 2 ml of 0.9% saline via the jugular vein. Group 2 (Ischemia–reperfusion group) were subjected to ischemia–reperfusion. Two milliliters of 0.9% saline was administered immediately prior to reperfusion period. Group 3 (Ischemia–reperfusion + tramadol group) were subjected to ischemia–reperfusion. A solution of 20 mg/kg tramadol [21] in 0.9% saline solution was administered, with a total volume of 2 ml.

2.2. Anesthesia

The rats were weighed and anesthetized using an intramuscular injection of ketamine hydrochloride10% and xylazine hydrochloride 2% (50 mg/kg and 10 mg/kg, respectively) [19].

2.3. Surgery

Surgery was performed at the Laboratory of Experimental Surgery, Science and Research College of Veterinary Medicine. The animals were placed on a board, in dorsal recumbency, with their thoracic and pelvic limbs immobilized with adhesive tape. The jugular vein was isolated and catheterized for the administration of heparin, tramadol and normal saline. After clipping, disinfecting with antiseptic povidone–iodine solution and draping, a skin incision was made on the medial surface of the left hind limb. After isolating of the femoral artery and vein from the surrounding tissues, the femoral artery was exposed.

Ischemia was induced by 2 h of femoral artery occlusion with a non-traumatic clamp and followed by 24 h of reperfusion. Prior to the occlusion of the femoral artery, 250 IU heparin [19] was administered via the jugular vein in order to prevent clotting. Rats were maintained in dorsal recumbency and kept anaesthetized (additional doses were given in case of necessity) throughout the duration of the ischemic period. Body temperature was maintained with a heating pad and monitored using a rectal thermometer. The vascular forceps were removed and the surgical site was routinely closed with 3/0 polypropylene sutures, following the ischemic period. Animals in the sham group underwent a surgical procedure similar to the other groups but the femoral artery was not occluded.

2.4. Specimen collection

At the end of the trial, blood samples were collected in heparinized vacutainer tubes and immediately transported to the laboratory on ice for serum studies. Moreover, the left gastrocnemius muscle was harvested, accomplished by incision in the posterior face of the leg, the gastrocnemius muscle being sectioned in its insertions to be submitted to histological and biochemical assays. The gastrocnemius muscles were washed three times in cold isotonic saline. Euthanasia was performed by using an overdose of pentobarbital (300 mg/kg) intraperitoneally.

2.5. Histological analysis

The histological tests of the gastrocnemius muscle were performed at the Department of Pathology, College of Veterinary Medicine. The Muscle samples were paraffin-embedded. Using standard techniques, paraffin sections were obtained at 5 μm, stained with hematoxylin and eosin, and studied under optical microscopy by a pathologist who was blinded to the experiment and data. Histological changes were scored on a scale from 0 to 3 where 0 = absence (<5% of maximum pathology), 1 = mild (<10%), 2 = moderate (15–20%), and 3 = severe (20–25%) [22]. A total of five fields of view from each muscle sample were randomly screened, and the mean was accepted as the representative value of the sample.

2.6. Serum biochemical dosing

Following values were determined for biochemical evaluation of the serum: creatine phosphokinase (CPK), Lactate dehydrogenase (LDH) and arterial blood gasometry (pH, pO_2, pCO_2 and HCO_3). Serum CPK and LDH assays were performed on a Hitachi System